TUBER PSEUDOEXCAVATUM sp. nov. A NEW SPECIES FROM CHINA COMMERCIALISED IN SPAIN, FRANCE AND ITALY WITH ADDITIONAL COMMENTS ON CHINESE TRUFFLES

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ABSTRACT: A new *Tuber* species is described from China which has been commercialised in Spain, France, and Italy. *Tuber pseudoexcavatum* is described and illustrated and additional comments on Chinese truffles are presented.

KEY WORDS: Tuher pseudoexcavatum, taxonomy, truffle market, legislation, Spain, France, China.

RÉSUMÉ: Des espèces de Tuber de Chine, commercialisées en Espagne et en France ont été étudiées. Une nouvelle espèce, T. pseudoexcavatum est proposée et entièrement décrite. Les truffes chinoises sont commentées.

MOTS CLEFS: Tuber pseudoexcavatum, taxonomie, marché de la truffe, législation, Espagne, France, Chine.

In Southwest China many truffle species have been commercially collected as food stuffs for a long time. Truffles are indeed an important resource in some regions of China. In particular, the large ascocarps of *Tuber sinense* (up to 10 cm in diam or more) are very abundant, while *T. pseudoexcavatum* and other species are more rare and have much smaller ascocarps.

The 1990's have seen the appearance of Chinese truffles at European markets particularly, *Tuber indicum* (Manjón *et al.*, 1995; Fourré *et al.*, 1996). In 1994, massive amounts of imported Chinese truffles began to arrive at French markets. This stimulated the French authors to investigate the identity of a number of Chinese *Tuber* species. In 1995 H. G. Fourré contacted and communicated with Dr. Wang Yun, a Chinese mycologist working on Chinese truffles in New Zealand. J. L. Riousset recognized that there were two Chinese truffle species which had been marketed in France, one is *Tuber sinense* Tao & Liu which is similar to *T. melanosporum* Vitt. and the other which was undescribed was similar to *T. excavatum* Vitt. (Fourré, pers. comm. 1995).

Some weeks later L.J. & G. Riousset received imported Chinese truffles from various French merchants. Most of these specimens were identified as *Tuber indicum* Cooke & Massee, but other specimens defied identification. These unidentifiable specimens were brown in color, subglobose and deeply excavate and also had eight ascospores per asci. In became apparent that it was a new species conspecific with the new species that

Dr. Wang found in Huidong Sichuan of China.

L. J. Riousset has concluded that this undescribed Tuber species is commonly

imported into France under the name Tuber indicum Cooke & Massee.

A similar story has been revealed for this species in Spain. Chinese truffles were first imported into Spanish markets in 1994 and their quantities has increased since. Interestingly most of these specimens imported in Spain came from France. As happened in France some of these Chinese truffles were identified as *T. indicum* (Manjón & al., 1995) and others were considered by the Spanish as a new species. When Dr. Moreno discussed the new species with Dr. L.J. & G. Riousset and G. Fourré, they agreed that both Spain and France had the same undescribed species which Dr. Yun had provisionally named *Tuber pseudoexcayatum*.

The presence of Tuber indicum Cooke & Massee in Italian markets has been

confirmed by Montecchi (1996).

MATERIAL AND METHODS

Ascocarps were collected from pine forest soils in Huidong, Sichuan, China and also in markets in Spain and France. Ascocarps were dried at room temperature and

deposited in herbariums as listed.

Scanning electron photomicrographs were made with a Zeiss DSM-950 microscope. Spore samples were rehydrated with 100% ammonium hydroxide for 30 min, then dehydrated in aqueous ethanol solutions (70%) for 1-1.5 h, before fixation in formaldehyde dimetilacetal. (after immersion in acetone for at least 2 h). Ascopores were subsequently critical point dried, mounted onto an aluminium stub, and coated with gold-palladium in a Polaron E-5000 sputter coater for 120 sec at 1.4 kV and 18 mA (argon atmosphere) creating a metal coating approximately 500 Å thick. Light photomicrographs were made with a Nikon Labophot microscope equipped with an automatic photographic system.

DESCRIPTION

Tuber pseudoexcavatum Wang, G. Moreno, L. J. Riousset, J. L. Manjón 🖩 G. Riousset, sp. nov. (Figs. 1-3)

Diagnosis: Ascocarpa subglobosa penitus excavata, usque ad 3,5 cm in diam, brunnea, verrucosa. Peridium bistratum. 290-470 mm crassum. Epicutis cellulis inflatis usque ad 20 mm, brunneis, subcutis hyphis hyalinis, intertextis, 3-5 mm in diam. Gleba solida alba demum canobruneola, venis albis. Asci sporis 1-8, subglobosis vel irregularis. Ascosporae ellipsoideae, (23-) 24-28 (-35) × (17-) 18-19 (22-) µm, spinoreticulatae.

Habitat in soils sylvarum montis usque ad 2000-2300 m supra mare, Sichuan provincia, 26 November 1989, Y. Wang & D. Ch Zhang, paratypus IFS89911. Imported from China: Yunnan, January 1995, L. J. Riousset Herbarium 01 14 03 95 holotypus.

isotypus in AH 18387.

Etymology: pseudoexcavatum in reference to the similarity of this species to Tuber excavatum.

Ascocarp subglobose, deeply excavate, brown to brown-orange, up to 3.5 cm in

diam, with a coarsely warted surface.

Peridium 290-500 μ m thick, composed of distinct outer and inner layers. Outer layer, 190-350 μ m thick (including warts), the outmost 3-5 layers of cells, subglobose, brown-red, 10-20 μ m of diam, thick-walled (1-3 μ m); just below these dark outermost cells lie pale subglobose cells which become smaller when they are closer to the inner layer (Figs. 1a, 1b). Inner layer 100-140 μ m thick, composed of hyaline, filamentous interwoven hyphae, 3-9 μ m in diam, thin-walled.

Gleba solid, white in youth but becoming gray-brown as spores mature, marbled with meandering, white veins of hyaline, interwoven hyphae, 3-5 µm in diam. Odour soft.

Asci subglobose, reniform or irregular, sometimes with short remains of croziers, $45-70\times60-85$ (-105) μm , with 1-8 clustered ascospores, inamyloid, indehiscent,

randomly embedded in gleba tissue. (Figs. 1d-h).

Ascospores ellipsoid, spinoreticulatae, variable in size, depending on the number of ascospores within an ascus (23-) 24-28 (-35) \times 16-19 (-22) μ m (excluding the ornamentation), on the average, (21-) 24-30 (-35) \times 16-22 μ m (1-spored asci), (23-) 25-30 (-34) \times (17-) 18-20 (-22) μ m (2-spored asci). Young ascospores hyaline, smooth, becoming dark brown, spinoreticulatae when mature. Spines up to 5 (-8) μ m tall and 1-2 mm thick at the base, hyaline in the youth becoming brown to dark brown, with broad basal connections tending to form a reticulation composed of variable meshes (Figs. 1d-h), but homogeneous in all the spore surfaces (Figs. 2a-f).

Habitat: in calcareous soils at 3-10 cm in depth under Pinus yunnanensis Franch

at elevations of 2000-2300 m, fruiting from August to November.

Material studied: Tuber pseudoexeavatum. collected from pine forest soils in Huidong, Sichuan, China, 26 November 1989, deposited in the Herbarium of Institute of Forestry & Soil Sciences. IFS 89912, IFS 89913, IFS 89914, IFS 89916, IFS 89919, IFS 89920, IFS 89922, leg. A.P. Li & Y.W. Li. IFS 89911, IFS 89910, IFS 89915, IFS 89917, IFS 89918; imported from China. January 1995, AH 18384 and AH 18385; imported from China: from pine forest soils in Yunnan. January 1995, L. J. Riousset Herbarium 01 14 03 95 holotypus, isotypus in AH 18387.

Material studied of other species: Tuber pseudohimalayense. Probably imported from China, in January of 1995. AH 18331 holotypus. Tuber himalayense isotypus AH 18383. Tuber indicum: Probably imported from China, in January of 1995. AH 18329

Remarks: Tuber pseudoexcavatum is characterized by ascocarps which are subglobose, deeply excavate, brown to brown-orange, with ■ coarsely warted surface, and

asci with 1-8 ascospores with spinoreticulatae ornamentation.

There are two other Tuber species with excavated ascocarps, T. excavatum Vitt. and Tuber mesentericum Vitt. The former differs by the smooth ascocarp surface, (1)3-5(6) spores per ascus and reticulated spores without spines. While T. mesentericum has dark black ascocarps, 1-5 (-6) spores per ascus and reticulated spores without spines.

Other Chinese Tuber species are T. sinense Tao & Liu (Tao & Liu, 1989), T. gigantisporum Wang & Li (Wang & Li, 1991), T. indicum Cooke & Massee (Cooke & Massee, 1892). T. himalayense Zhang & Minter (Zhang & Minter, 1988) and. T. pseudohimalayense G. Moreno, Manjón, Díez & Garcia-Montero (Moreno & al. 1997). Tuber pseudoexcavatum differs from these other Chinese species by the excavated ascocarp and the 8-spored asci.

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LITERATURE CITED

FOURRÉ G., RIOUSSET L. J. & RIOUSSET G., 1996 — Ces "truffes de l'Inde" qui nous arrivent de Chine. Bulletin de la fédération des associations mycologiques méditterranéennes 9: 3-21.

MANJÓN J. L., MORENO G., DÍEZ J. & GARCÍA-MONTERO L. G., 1995 - Tuber indicum Cooke & Massee en España: Una especie comercializada con características similares a nuestra trufa negra. Boletin de la sociedad micologia. Madrid 20: 301-304.

MONTECCHI A., 1996 - Tuber indicum Cooke & Massee, XXI Mostra Reggiana del fungo. Il

Fungo suppl. 3 (14): 27-30.

MONTECCHI A. & LAZZARI G., 1993 - Atlante fotografico di funghi ipogei. Associazione Micologica Bresadola. Centro Studi Micologici. Vicenza.

MORENO G., MANJÓN J. L., DÍEZ J., GARCÍA-MONTERO L. G. & DI MASSIMO G., 1997 -Tuber pseudohimalayense sp. nov. an asiatic species commercialized in Spain, similar to the "Perigord" truffle, Mycotaxon 63: 217-224.

TAOK. & LIUB., 1989 - A new species of the genus Tuber from China. Journal of Shanxi University

(Nat. Sci. Ed.) 12 (2): 215-218.

- TRAPPE J. M., JUMPPONEN A. M. & CÁZARES E., 1996 Nats truffle and truffle-like fungi 5: *Tuber lyonii* (= *T. texense*), with a key to the spiny-spored *Tuber* species groups. *Mycotaxon* 60: 365-372.
- UECKER F. A. & BURDSALL H. H., 1977 Tuber spinoreticulatum, a new truffle from Maryland. Mycologia 69: 626-630.
- WANG Y. & LIZ. P., 1991 A new species of *Tuber* from China. Acta Mycologia Sinica 2: 12-15. ZHANG B. C. & MINTER D. W., 1988 Tuber himalayense sp. nov. with notes on Himalayan truffles. Transactions of the british mycological society 91(4): 593-597.
- ZHANG D. Ch. & WANG Y., 1990 Study on Chinese truffle and its ecology. Edible Fungi of China 9 (2): 25-27.

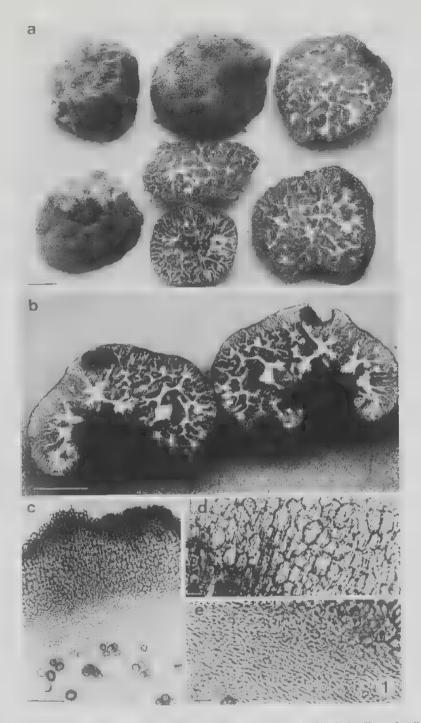


Fig. 1. — Tuher pseudoexcavatum (Holotypus): a-b. ascocarps and glebal detail; c. detail of the external peridium; d. thick-walled, globose cells of the external peridium; e. plectenquimatic cells of the internal peridium. Bars: a, b = 1 cm; c = 100 μ m; d, e = 5 μ m.

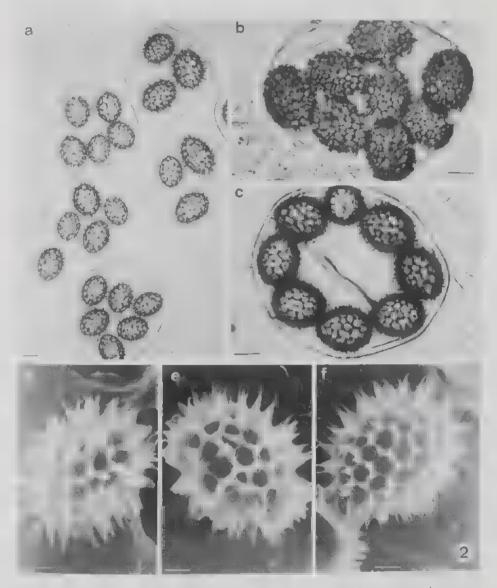


Fig. 2. — *Tuber pseudoexcavatum (Holotypus)*: a-c. asci with 3, 5, 6 and 8 ornamentated ascospores; d-f. SEM micrographs of ascospores, showing the detail of the ornamentation. Bars: a-c = $10~\mu m$: d-f = $5~\mu m$.

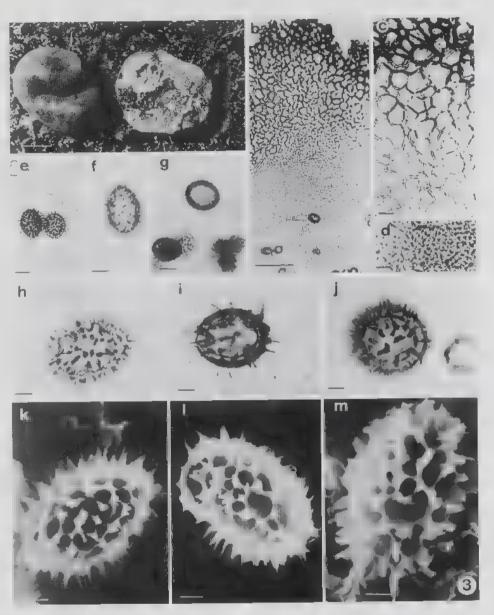


Fig. 3. — Tuber pseudoexcavatum (AH 18384): a. ascocarp and detail of the gleba; b. detail of the external peridium; c. thick-walled, globose cells of the external peridium; d. cells plectenquimatic of the internal peridium; e-g. asci with 1 and 2 ascospores; f, h-j. ascospores with spines with broad basal connections to form a reticulum; k-m. SEM micrographs of ascospores, showing the detail of ornamentation. Bars: a=1 cm; $b=100~\mu m$; c-g=10 μm ; h-m=5 μm .